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Alteration of Oligosaccharide Biosynthesis by Genetic Manipulation of Glycosyltransferases^a

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INTRODUCTION

Carbohydrate chains can directly mediate or modulate the function of glycoproteins in diverse biological processes.¹ The ability to manipulate the oligosaccharide structures of glycoproteins in order to alter their biological properties would be of obvious value. This is particularly relevant with regard to biologically important molecules such as growth factors, hormones, and other therapeutic agents that are being produced in cultured cells. Altering the sugar chains of these glycoproteins may improve their therapeutic value by increasing their efficacy, altering their circulatory half-lives, and/or increasing their target specificity. In addition, altered glycosylation of cell-surface components may provide insight to the precise roles that cell-surface glycoconjugates play in processes such as migration, adhesion, development, and malignancy.

Several methods have evolved to alter glycosylation in cells. These have included the use of reagents that inhibit glycosylation as well as inhibitors of glycosylation processing.² These inhibitors have been widely used to study the sugar chains of glycoconjugates, but many of these reagents are toxic to cells, and in some instances, their effects are only partial. Another approach has been to use mutagenized cells that are resistant to the toxic effects of specific lectins due to deficiencies in corresponding glycosylation reactions.³ One limitation of this approach is that mutants are not generated at each step of the biosynthetic pathway. In addition, both of these approaches are of limited use for obtaining large, complex oligosaccharides, inasmuch as they result in the formation of incomplete or truncated carbohydrate structures.

One method that overcomes these limitations and that allows one to selectively manipulate oligosaccharide structure is to express cloned genes for glycosyltransferases into mammalian cells. As more genes for these enzymes are cloned, the possibilities for altering the biosynthetic pathways of oligosaccharide in cells could be substantial. It is this new approach that will be the focus of this review.

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TRANSFERASES

alized to the endoplasmic reticulum and these enzymes catalyze the transfer of sugars from intermediates to mono- and oligosaccharide chains. Different glycosyltransferases have been identified, and many of these enzymes share common subunit topology. The topology and domain structure of these enzymes, however, are remarkably similar in that they all contain short cytoplasmic domains and a transmembrane domain. Availability of cDNAs for many of these enzymes has allowed them to be transfected into host cells and to subsequently

allow genetic manipulation of glycosyltransferases. Expression of glycosyltransferases that are not endogenous (exogenous glycosyltransferases), and overexpression of cells containing endogenous activities of glycosyltransferases. The first approach has been the transfection of cells for alterations in glycosylation is relatively simple. To remove oligosaccharides on the cell surface, one can use a second approach, on the other hand, remove oligosaccharides because increasing the levels of glycosylation can alter different types, but rather, different

MANIPULATION OF GLYCOSYLTRANSFERASE EXPRESSION

Glycosyltransferases

Expression of exogenous glycosyltransferases has been used for modification, such as fucosylation and sialylation. Studies that have used this approach to modify

glycosyltransferase catalyzes the transfer of fucose (Fuc) from

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fucose to galactose (Gal) residues, forming the H antigen. Similar to the studies with α 1,2 FT into COS-1 cells, which lead to the formation of H blood group antigen.¹⁰

C. α 1,3 Galactosyltransferase. Transfection of UDP-Gal to terminal Gal residues into CHO cells results in the formation of H antigen.¹¹ Furthermore, the expression of α 1,3 Galactosyltransferase in terminal sialylation of sugar chains results in competition between different glycosyltransferases.

D. α 2,6 Sialyltransferase (α 2,6 ST). Transfection of CMP-SA to terminal Gal residues into CHO cells results in the formation of H antigen.¹² As was the case for example, between the transfected sialyltransferase and endogenous SA in an α 2,3 linkage to Gal.

E. β 1,3 Galactosyltransferase (β 1,3 GT). Transfection of GlcNAc residues in the biosynthesis of oligosaccharide chains. This class of oligosaccharide chains is of importance because it serves as the core fucose. Transfection of the cDNA encoding the biosynthesis of type 1 N-glycans. In addition, many of these chains were found to be in contrast to the studies described above. Expression of glycosyltransferase during elongation) can also alter

Endogenous

To date, the only example of glycosyltransferase is that of β 1,4 Galactosyltransferase, which catalyzes the transfer of Gal from UDP-Gal to GlcNAc in the biosynthesis of type 2 (Gal β 1,4 GlcNAc) complex oligosaccharides. β 1,4 Galactosyltransferase in that one gene encodes two similar

fucose to galactose (Gal) residues, resulting in the formation of the H blood group antigen. Similar to the studies with $\alpha 1,3$ FT, the introduction of the cloned gene for $\alpha 1,2$ FT into COS-1 cells, which lack this enzymic activity, results in the formation of H blood group antigen.¹⁰

C. $\alpha 1,3$ Galactosyltransferase ($\alpha 1,3$ GT) catalyzes the transfer of Gal from UDP-Gal to terminal Gal residues of oligosaccharide chains. Transfection of $\alpha 1,3$ GT into CHO cells results in the expression of $\alpha 1,3$ Gal-containing oligosaccharides.¹¹ Furthermore, the expression of this enzyme results in a concomitant decrease in terminal sialylation of sugar chains in these cells. Thus, this study also shows that competition between different glycosyltransferases can affect glycosylation *in vivo*.

D. $\alpha 2,6$ Sialyltransferase ($\alpha 2,6$ ST) catalyzes the transfer of sialic acid (SA) from CMP-SA to terminal Gal residues. Transfection of the cDNA encoding this protein into CHO cells results in the expression of appropriately sialylated sugar chains.¹² As was the case for example C above, there is competition for substrates between the transfected sialyltransferase and a similar endogenous enzyme that adds SA in an $\alpha 2,3$ linkage to Gal.

E. $\beta 1,3$ Galactosyltransferase ($\beta 1,3$ GT) catalyzes the transfer of Gal to terminal GlcNAc residues in the biosynthesis of type 1 (Gal $\beta 1,3$ GlcNAc) *N*-acetylactosamine chains. This class of oligosaccharides is of particular biological and structural importance because it serves as the core structure for various blood group activities. Transfection of the cDNA encoding this enzyme into human colonic cells results in the biosynthesis of type 1 *N*-acetylactosamine-containing oligosaccharides.¹³ In addition, many of these chains were further modified with SA and Fuc residues. In contrast to the studies described above, this study is unique in showing that the expression of glycosyltransferase acting early in oligosaccharide biosynthesis (*i.e.*, during elongation) can also alter glycosylation.

Endogenous Glycosyltransferase

To date, the only example of over-expression of an endogenously expressed glycosyltransferase is that of $\beta 1,4$ galactosyltransferase ($\beta 1,4$ GT). $\beta 1,4$ GT catalyzes the transfer of Gal from UDP-Gal to terminal GlcNAc residues in the biosynthesis of type 2 (Gal $\beta 1,4$ GlcNAc) *N*-acetylactosamine cores of all *N*-linked complex oligosaccharides. $\beta 1,4$ GT is unique among the cloned glycosyltransferases in that one gene encodes two similar forms of the enzyme that differ by an additional 13 amino acids at the cytoplasmic N-terminus of the long form that is not present in the short form of the enzyme.^{14,15} Both the long and the short form are localized primarily in the Golgi complex. However, the long form of GT is also targeted to the plasma membrane,¹⁵ where it associates with the cytoskeleton and functions as a cell-adhesion molecule.¹⁶

Because of the central role this enzyme plays in the biosynthesis of *N*-acetylactosamine-containing oligosaccharides, the effects of overexpressing this enzyme were explored in detail. Transfection of the cDNAs encoding the two forms of $\beta 1,4$ GT into F9 embryonal carcinoma cells expressing endogenous enzyme results in a threefold increase in total $\beta 1,4$ GT activity compared to control cells.¹⁷ Analysis of [³H]Gal-labeled glycoproteins and glycopeptides by a variety of methods revealed

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differences in glycosylation. Similar animal-associated membrane glycoprotein glycosylation between the transfected and control cells. The addition of exogenous β 1,4 GT to affect glycosylation was as much as an excess of substrate was and exogenous GT. The transfected GT activity, and, more importantly, were elevated. Thus, in these cells, β 1,4 GT is not rate

ABSTRACT

structures through genetic manipulation of cells. It is apparent that this technique has been used to study the effect of enzyme structure when an exogenous enzyme is added. In this enzyme is responsible for a terminal sugar, this study has examined the effects of overexpression, in which there was no detectable difference. There are still other key regulatory biosynthetic enzymes, such as β 1,3 GlcNAc transferase, whose overexpression of these enzymes are required for the synthesis of polymers of *N*-acetylglucosamine disaccharides. The expression of this gene encoding GlcNAc transferase V has been studied and characterization of the resulting

polysaccharide structures could involve the addition of the enzyme into cells to ensure the availability of substrate. The disruption of specific glycosyltransferases could be used to eliminate competing pathways.

Activity is directly dependent upon the presence of other factors also contribute to glycosylation. A glycoprotein through the endoplasmic reticulum processing glycosidases, the availability

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